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The efficiency of some natural alternatives in root-knot nematode control

Abstract

Plant extracts are, nowadays, extensive used as environment friendly ways for biological control of parasitic pests, including the root-knot nematodes, instead of using chemical pesticides. Therefore, the aim of this study was to analyze leaf and root extracts nematicidal activities of four selected medicinal plants (i.e., *Azaddirachta indica*, *Moringaoleifera*, *Lantana camara*, and *Glycyrrhizaglabra*) against the root-knot nematode; *Meloidogyne* spp. Roots of *G. glabra* and leaves of *A. indica*, *M. oleifera*, and *L. camara* were collected from different sites in Fayoum Governorate. Roots and leaves were air-dried, powdered and then extracted by ethanol 95% for *L. camara* and *G. glabra* or by petroleum ether for *A. indica* and *M. oleifera*. The nematode eggs were exposed to the different extracts at different concentrations (i.e., 500, 1000, 2000, 4000ppm) for 24, 48 and 72h. Results showed that all four plant extracts caused significant decreases in egg hatching, but to varying degrees. *A. indica* extract was the most effective in preventing egg hatching, followed by *M. oleifera* extract. There was a gradual decrease in egg hatching with increasing the extract concentration and the duration of exposure. As the most effective, the crude extract of *A. indica* was analyzed by using GC/MS for the effective ingredients and found to be included alkaloids, flavonoids, saponins, amides including benzamide and ketones, and others, which showed effectiveness in preventing the egg hatching of the root-knot nematode; *Meloidogyne incognita*.

Keywords: extract, *Meloidogyne* spp., egg hatchability, mortality, GC/MS

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Introduction

Nematodes are found in a wide variety of habitats. Free-living nematodes live in the soil, in freshwater, marine sands and muds. In soil, they are important components of nutrient turnover. Other nematodes are parasites of almost every species of animal, humans, plant and they cause enormous social and economic damage.[1] Phytoparasitic nematodes parasitize plants to seek suitable food. This food source is basically planted cell contents. Thus a plant response to parasitism is the reaction to the cellular feeding of the nematode.[2] Most phytoparasitic nematodes infect plant roots and some species have evolved sophisticated interactive relationships with host cells to sustain a sedentary parasitic habit.[3] Plants carry a wide range of microorganisms in their phyllosphere and Rhizosphere which not only cause a large variety of diseases but also control of pathogens.[4] Nematodes have an important niche in agro-ecosystem, causing a reduction in plant productivity and growth. Root-knot nematodes (*Meloidogyne* spp.) are very common and the most important nematode species of greenhouse-growing plants. Indiscriminate use of chemical nematicides to control nematode causes great injuries to human being, animal, vegetation and to the environment as a whole due to their non-target effect, hazardous nature besides they are expensive. So with the increasing awareness of possible deleterious effects of the chemicals, biological controls of plants pathogen have received considerable attention.[5] The management of these nematode-parasites has little chance of success and is uneconomical because they live in the soil and feed on the internal plant tissues. Preventing the introduction of nematodes with planting material, seeds, or soil, using rotation and mixed cropping with the poor host, using nematode resistant varieties or rootstocks, and lowering nematode populations through nematicides are some of the most frequently used strategies.[6] Until recently, methyl bromide was widely used to manage nematodes and other soil-borne pathogens in high-value horticultural crops. However, concerns on its impact on environment necessitate the ban or revoke of this methyl bromide in 2005 for its gas emission and global warming. Although nematicides are effective in nematode management, it discourages users because of their high costs, non-availability at the time of need, the hazards they pose on human as well as on non-target organisms.[7] Other options for the management of root-knot nematodes become imperative and there is an increasing interest in non-chemical nematode management strategies.[8] Extract from certain plants is used to control certain nematode because environmental consideration and costs of nematicides dictate that other methods of control may be investigated, on alternative method is the use of antagonistic plants in rotation with or inter planted with crop plants. Certain medicinal plant extracts and their constituent were experimentally used for such aim.[9–11] The current study was designed to evaluate the potential beneficial effects of some plant extracts such as lantana (*Lantana camara*), neem (*Azadirachta indica*), moringa (*Moringaoleifera*), and liquorices (*Glycyrrhiza glabra*) on controlling the root-knot nematode (*Meloidogyne* spp.) through their toxic effects on egg hatchability.

Material and methods

Plant material used in the experiment

As shown in (Table 1), plant materials of *Moringaoleifera* and neem were collected from mature plants grown at Demo Experimental Farm of Faculty of Agriculture, Fayoum University, and Fayoum, Egypt. In addition, *Lantana camara* leaves were collected from gardens of Faculty of Agriculture, Fayoum University, and Fayoum, Egypt. However, liquorice roots were collected from Anonymous fields located in Abshwai district, Fayoum Governorate, Egypt.

Table 1 Information about the four plant species used in the present study

English Name	Scientific Name	Family	Plant part used	Reference of previous use
Lantana	*Lantana camara*	Verbenaceae	Leaves	Tayeet al.,[40]
Neem	*Azadirachtaindica*	Meliaceae	Leaves	Tayeet al.,[40]
Moringa	*Moringaoleifera*	Moringaceae	Leaves	Sowleyet al.,[39]
Liquorice	*Glycyrrhizaglabra*	Fabaceae	Roots	Sardariet al.,[38]

Preparation of plant extracts

Plant leaves were plucked from their branches and spread on polythene sheets on benches in the laboratory for ten days to air dry. The dried materials were ground to fine particles using a blender. An amount of 400ml ethanol (95%) (*L. camara* and *G. glabra*)and Petroleum Ether (*A. indica*and *M. oleifera*) were added to 100g of ground plant material and shaken on a rotary in a shaker at 120rpm for 24 hours. The solution was filtered through muslin cloths then through Whatman No. 1 filter paper and the material was vacuumed in a rotary evaporator at 40°C to obtain organic crude extracts (solvent is eliminated).[12] Extracts were used at 4000, 2000, 1000 and 500ppm concentration that obtained by the dilution with distilled water.

Extraction of nematode eggs

Eggs were obtained from a culture of nematode infected roots of tomato, root pieces containing egg masses were cut into small pieces and placed in a container of 500 ml capacity with 200 ml of 0.5% Clorox (sodium hypochlorite, NaOCI) solution shaken vigorously by hand for 4 min.[13] This was done in order to digest the gelatinous matrix encasing the eggs. The solution was then poured through two nested sieves, 200- mesh (75µm) and 500mesh (25µm). Eggs in the 500 mesh sieve were washed free of NaOCI solution with a slow stream of cold tap water into a container previously marked to contain 1 L. The cut roots in the original container were washed twice with water to obtain additional eggs. The collected eggs were topped with water to obtain the egg-water suspension for *in vitro* studies.

Counting of root-knot nematodes eggs

Number of eggs in aqueous suspension was determined by using a stereo microscope. One milliliters of the egg-water suspension was pipette after bubbling air through the suspension for homogeneity and dispensed into a counting tray. Counting was done two times and the mean number of eggs/ml estimated.

Hatchability test

Eggs were collected by the method of Hussey and Barker.[13] A suspension of eggs in water was prepared. 1 ml of egg suspension (100±10 eggs/ml) and 5 ml of leaf or root extract was transferred in Petri dishes and kept at room temperature. Each treatment was 3-time replicated. The Petri dishes containing 1 ml egg suspension and 5 ml water served as control. After 24, 48, 72 hours of exposure, the number of hatching eggs was counted under an inverted microscope.

Gas chromatography-mass spectrometry (GC/MS) analysis

The GC column was a 30m (0.25mm i.d., film thickness 0.25µm) HP-5MS (5%diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 50°C for 2 min, then programmed to 280°C at 6°C/min and held at this temperature for 2min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70eV ionization energy. The sector mass analyzer was set to scan from 40 to 400amu for 5s. These data were obtained from environmental and food pollutants laboratory at Faculty of Agriculture, Fayoum University.

Results

Effect of exposure time and inhibition concentration (IC)

Regarding the effect of some plant extracts on egg hatching of root-knot nematode after 72h, data in (Table 2) and (Figure1) (Figure2) show that toxicity of extract IC_{50} (Inhibition Concentration, 50%), IC_{90} and slope value was calculated. It shows that neem extract is highly effective against egg hatching being the IC_{50} scored 202.55ppm followed by moringa extract IC_{50} conferred 497.55ppm, while the least effective extract against egg hatching was liquorices extract IC_{50} granted 1479.15ppm. Consequently, neem extract caused 64, 76, 84 and 89% inhibition of egg hatching on root-knot nematode at the concentrations of 500, 1000, 2000 and 4000ppm, respectively. In contrast, the liquorices extract at the concentrations of 500, 1000, 2000 and 4000ppm caused the inhibition % of egg hatching of 15, 44, 63 and 74%, respectively at 72h.

Table 2 Effect of some plant extracts on egg hatching (%) of root-knot nematode (*Meloidogyne* spp.) after 72 hours exposure to the extracts.

Extract	Concentration(ppm)				IC50(ppm)	95% Confidence limits		IC90(ppm)	Slope ± SE
	500	1000	2000	4000		Lower	Upper		
Neem	64*	76	84	89	202.6	46.3	374.7	4183.2	0.97±0.22
Moringa	49	66	77	86	497.6	268.3	686.2	5600.5	1.22 ±0.21
Lantana	33	55	70	79	920.8	693.5	1145.8	7741.2	1.39 ± 0.20
Liquorice	15	44	63	74	1479.2	651.1	3547.9	7621.5	1.80± 0.21

*Inhibition of egg hatchability (%)

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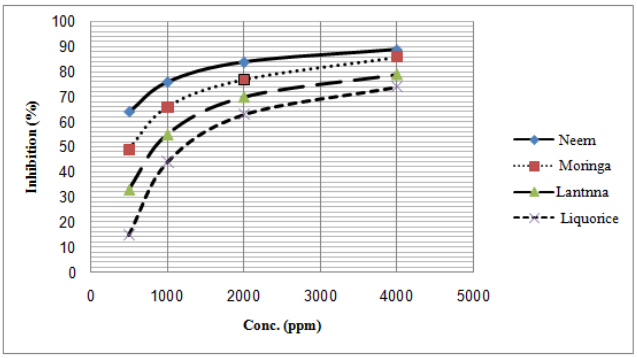

Figure 1 Effect of some plant extracts on egg hatching of root-knot nematode (*Meloidogyne* spp.) after 72 hours.

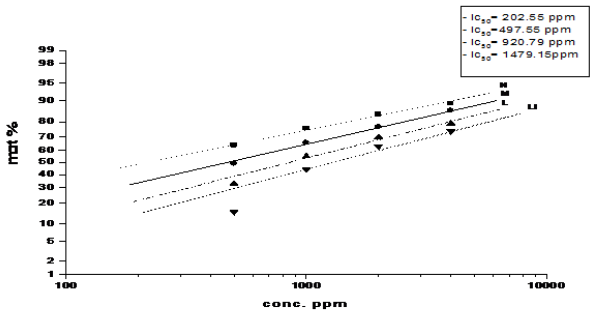

Figure 2 Effect of some plant extracts on egg hatching of root-knot nematode (*Meloidogyne* spp.) after 72 hours.

Effect of plant extract, concentration and exposure time

The mean performance of plant extract and the effect of the extract concentration and exposure time to extract on egg hatching of root-knot nematode are shown data in (Table 3). Data show that the egg hatching (%) was recorded the highest value with the extract of *Glycyrrhiza glabra* followed by *Lantana camara* extract, then *Moringaoleifera* extract, and the lowest value was recorded with the extract of *Azadirachta indica.* The egg hatching (%) was progressively reduced with increasing the extract concentration from 500 to 4000ppm. In contrast, the egg hatching (%) was progressively increased with increasing the exposure time from 24 to 72h.

Table 3 Mean performance (± SE) of plant extract, concentration and time of egg hatching on *Meloidogyne* spp.

Plant	Means(%)±SE	Conc.(ppm)	Means(%)±SE	Time (h)	Means(%)±SE
Azadirachtaindica	32.1±2.9 d	0	80.8±2.1 a	24	34.3±1.7c
Moringaoleifera	38.5±2.7 c	500	48.3±2.2 b	48	44.7±2.5b
Lantana camara	46.0±2.6 b	1000	36.3±1.4 c	72	48.7±2.9a
Glycyrrhizaglabra	53.6±2.7 a	2000	24. ±1.0 d	-	-
-	-	4000	16.5±0.7 e	-	-

Interactive effect of plant extract and its concentration

The interactive effect of plant extract and its concentration on egg hatching of root-knot nematode is presented in (Table 4) and (Figure 3). Neem extract significantly decreased egg hatching of root-knot nematode. The percentages of reductions of egg hatching were 59.01, 73.03, 81.83 and 87.34% by application of neem extract (*Azadirachtaindica*) at the concentrations of 500, 1000, 2000 and 4000ppm, respectively compared to the control (water). Egg hatching of root-knot nematode was significantly decreased egg hatching by moringa extract (*Moringaoleifera*) application. The percentages of reductions of egg hatching were 42.36, 61.48, 74.27 and 83.77% of the moringa extract at the concentrations of 500, 1000, 2000 and 4000ppm, respectively compared to the control. Lantana extract (*Lantana camara*) significantly decreased egg hatching of root-knot nematode. The percentages of reductions of egg hatching were 23.38, 49.24, 66.16 and 76.47% by application of lantana extract at the concentrations of 500, 1000, 2000 and 4000ppm, respectively compared to the control. Egg hatching of root-knot nematode was significantly decreased egg hatching by liquorices extract (*Glycyrrhizaglabra*) application. The percentages of reductions of egg hatching were 3.71, 36.45, 57.36 and 70.69% by application of liquorices extract at the concentrations of 500, 1000, 2000 and 4000ppm, respectively compared to the control.

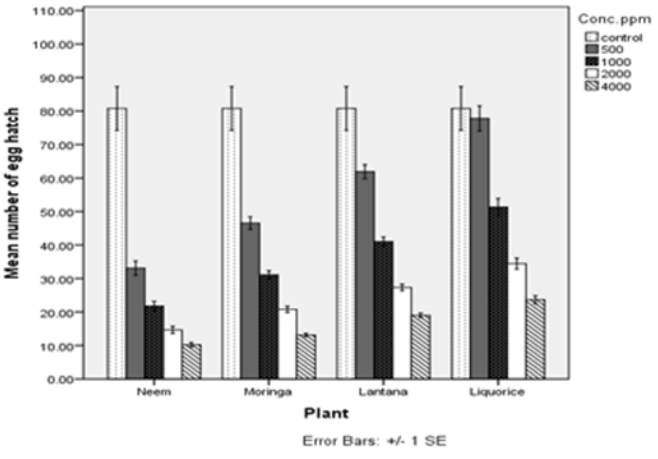

Figure 3 Mean performance of interaction between plant extract and the concentration of egg hatching on *Meloidogynes* pp.

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Table 4 Mean performance (±SE) of interaction between plant extract and concentration on egg hatching of *Meloidogyne* spp.

Plant	Concentration(ppm)	Means(%)±SE
Azadirachtaindica	0	80.8*±4.4a
	500	33.1±1.3f
	1000	21.8±0.9hi
	2000	14.7±0.6j
	4000	10.2±0.4k
Moringaoleifera	0	80.8±4.4a
	500	46.6±1.3d
	1000	31.1±0.8f
	2000	20.8±0.7hi
	4000	13.1±0.4jk
Lantana camara	0	80.8±4.4a
	500	61.9±1.4b
	1000	41.0±1.0e
	2000	27.3±0.7g
	4000	19.0 ± 0.5i
Glycyrrhizaglabra	0	80.8 ± 4.4a
	500	77.8 ± 2.7a
	1000	51.3 ± 1.9c
	2000	34.4 ± 1.2f
	4000	23.7 ± 0.9h

*Inhibition of egg hatchability (%)

Interactive effect of plant extract and exposure time:

(Table 5) (Figure 4) illustrate the interactive effect of plant extract and exposure time on egg hatching of root-knot nematode, the most effective extract at decreasing the egg hatching is *Azadirachta indica* followed by *Moringaoleifera*, and the lowest effective extract at inhibiting the egg hatching is *Glycyrrhiza glabra*.

Table 5 Mean performance and S.E. of interaction between plant extract and time of egg hatching on *Meloidogyne* spp.

Plant	Time (h)	Means(%)±SE
Azadirachtaindica	24	25.1*±3.3j
	48	33.9±5.2h
	72	37.3±6.1g
Moringaoleifera	24	30.7±3.3i
	48	40.5±4.9f
	72	44.3±5.7e
Lantana camara	24	37.3±3.0g
	48	48.2±4.6d
	72	52.5±5.3c
Glycyrrhizaglabra	24	43.9±3.3e
	48	56.2±4.6b
	72	60.7±5.3a

*Inhibition of egg hatchability (%)

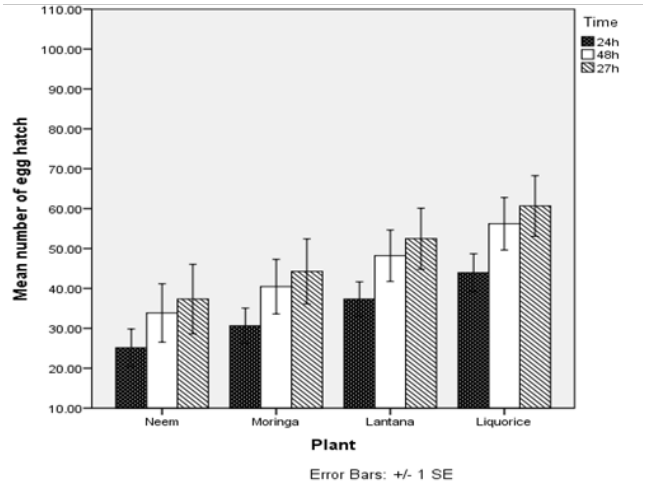

Figure 4 Mean performance of interaction between plant extract and time of egg hatching on *Meloidogyne* spp.

Interactive effect of plant extract, concentration and exposure time:

(Table 6) (Figure 5) reveal the interactive effect of plant extract, concentration and exposure time on nematode egg hatching Under the application of plant extract concentration for different periods, the lowest effective plant extract concentration was 500ppm, which gave the least inhibition, followed by 1000ppm. The highest effective extract concentration was 4000ppm, conferring the lowest egg hatching.

Table 6 Mean performance (± SE) of interaction between concentration and time of egg hatching on *Meloidogyne* spp.

Concentration(ppm)	Time(h)	Means(%)±SE
0	24	56.7±0.9d
	48	85.7±0.7b
	72	100.0±0.0a
500	24	47.7±3.2e
	48	57.2±3.7cd
	72	59.7±3.9c
1000	24	31.6±2.1g
	48	37.9±2.5f
	72	39.4±2.6f
2000	24	21.1±1.5i
	48	25.4±1.7h
	72	26.4±1.8h
4000	24	14.3±1.0k
	48	17.3±1.2jk
	72	17.9±1.2j

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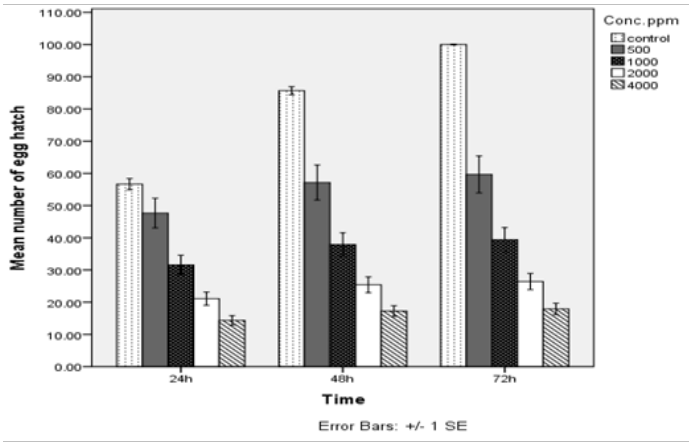

Figure 5 Mean performance of interaction between concentration and time of egg hatching on *Meloidogyne* spp.

Effect of plant extract at different periods:

(Table 7) (Figures 6–8) show the effect of plant extract on hatching of root-knot nematode at different periods. The four plants; neem, moringa, lantana and liquorices were tested at different concentrations (500, 1000, 2000 and 4000ppm) for egg hatching of root-knot nematode. The results show a gradual decrease in egg hatching with increasing the concentration of each extract. The increase in exposure period and an increase of the concentration also decrease of egg hatching. After 24 h application of the extracts on root-knot nematode eggs, the mean egg hatching ranged from 8.67 to 67.67%. The highest egg hatching was observed in the *G. glabra*, whilst the lowest was observed with the *A. indica*. At 48 h, mean egg hatching was ranged between 11.00 and 85.67%. The highest egg hatching was found in the control, whilst the least hatch was in the *A. indica* extract. In addition, at 72h mean egg hatching was ranged between 11.00 to 100 %. The highest egg hatching was observed in the control treatment, whilst the lowest hatching was found out in the *A. indica* extract. The most effective plant extract inhibition of egg hatching was *A. indica* extract at the concentration of 4000ppm, which conferred the lowest egg hatching.

Table 7 Effect of some plant extracts on hatching of *Meloidogynes* pp. egg at different periods.

Treatment	Conc.(ppm)	Mean number of egg hatching (%)		
		24h	48h	72h
Azadirachtaindica	500	28.7±1.6n_q	34.7±1.9j_m	36.0 ±2.1jk
	1000	19.0±1.1t_z	22.7±1.4r_u	23.7 ±1.4q_u
	2000	12.7±1.0a_c	15.3±1.0x_b	16.0 ±1.1w_b
	4000	8.7±0.5c	11.0±0.7bc	11.0 ±0.7bc
Moringaoleifera	500	40.0±1.1hij	48.7±1.6fg	50.7 ±1.6ef
	1000	27.0±0.7o_r	32.7±0.9k_o	33.7 ±0.9k_n
	2000	18.0±0.7u_a	21.7±0.9r_w	22.7 ±0.9r_u
	4000	11.3±0.5bc	13.7±0.2z_c	14.3 ±0.5y_c
Lantana camara	500	54.0±0.5d_f	64.3±0.7c	67.3 ±0.7c
	1000	35.7±0.3j_l	42.7±0.3hi	44.7 ±0.3gh
	2000	23.7±0.3q_u	28.7±0.3n_q	29.3 ±0.3m_p
	4000	16.7±0.3w_b	19.7±0.3t_y	20.7 ±0.3s_x
Glycyrrhizaglabra	500	67.7±3.4c	81.0±3.8b	84.7 ±4.1b
	1000	44.7±2.5gh	53.7±2.9d_f	55.7 ±2.9de
	2000	30.0±1.6l_p	36.0±1.6jk	37.3 ±1.8i_k
	4000	20.7±1.0s_x	24.7±1.4 p_t	25.7 ±1.4p_s
Control	0	56.7±2.2d	85.7±1.5b	100.0 ±0.0a

Data are means ±S.E. different lower or upper letters in a column indicate significant differences between the treatments at $P \leq 0.05$.

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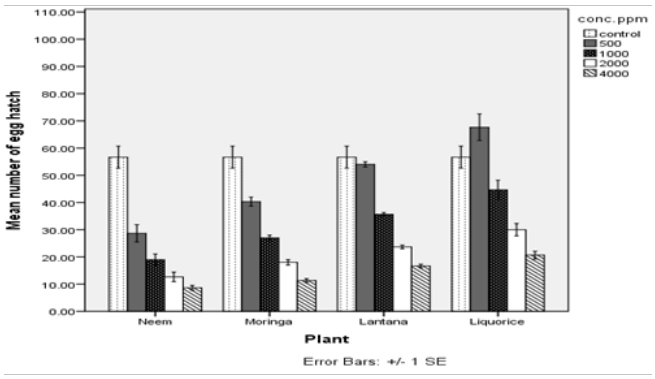

Figure 6 Effect of some plant extract on hatching of *Meloidogyne* spp. egg at 24.

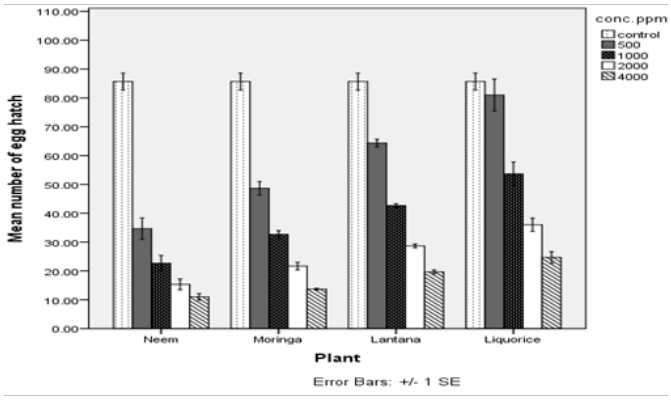

Figure 7 Effect of some plant extract on hatching of *Meloidogynes* pp. egg at 48h.

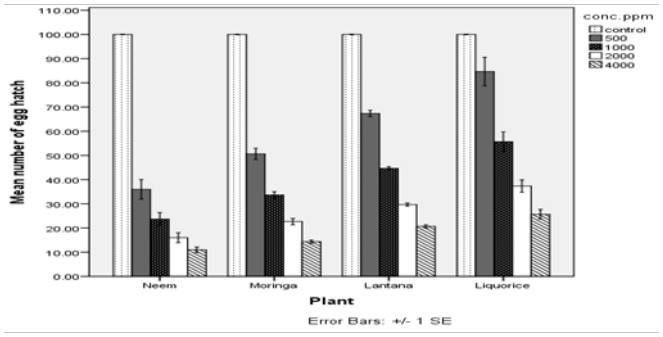

Figure 8 Effect of some plant extract on hatching of *Meloidogyne* spp. egg at 72 h.

Active chemical compounds of neem leaf extract

As the most effective extract inhibiting nematode egg hatching, the GC/MS analysis (Figure 9) of the leaf extract of neem showed the following active chemical compounds: Hexane, 2, 4-dimethyl; Hexane, 2,2,5-trimethyl;Cyclohexane, 2,4-diethyl-1-methyl-; Methylbicyclo [4.2.0] octane; Methallylcyclohexane; Cyclohexane ethyl-; Heptane, 2,6-dimethyl; Cyclohexane, 1,2,4-trimethyl-, (1α,2β,4β)-; Trans-1,2-Diethyl cyclopentane; Octane, 4- methyl-; Heptane, 2,3-dimethyl-; Benzene, 1,3-dimethyl-; Cyclohexane, 1,1,2-trimethyl-; Cyclopentane, 1-methyl-2-propyh-; 1-Ethyl-4-methylcyclohexane; p-Xylene; Nonane; Cyclohexane, 1-ethyl-4-methyl-, cis-; Benzene (1-methylethyl)-; Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2- methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Dodecane; -; o-Xylene; Mesitylene; and Naphthalene, decahydro-, trans-.

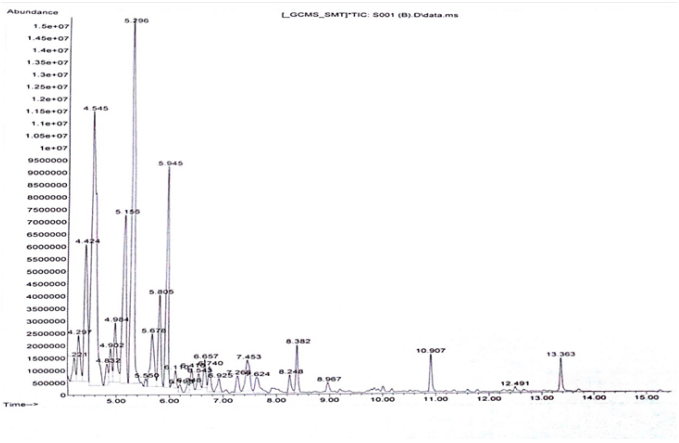

Figure 9 Chromatogram obtained from the GC-MS with the extract of *Azadirachta indica* leaves.

Discussion

The recent approach in nematode control is direct method towards the possibility of reducing populations of plant-parasitic nematodes in soil by using natural substances extracted from some plants. Such methods don't lead to the disturbance of the biological balance of nature. Utilization of antagonistic plants or their byproducts is of common use all-over the world for avoiding hazards of the traditional chemical nematicides. The use of certain plant extracts for controlling plant-parasitic nematodes has been increased in the recent years.[14–17] The plant extracts which tested in this study found in most cases to have an antagonistic action and a higher nematicidal activity against root-knot nematode. So, they undoubtedly contain natural nematotoxic constituents that able to inhibit the nematode egg hatching. In a study conducted by Hussaini et al.,[18] it has been reported that leaf extracts of 11 plant species inhibited egg hatching and caused 90% larval mortality in *M. incognita*, *M. javanica,* and *M. arenaria*. Our results are in parallel line with the results of Hussaini et al.,[18] In addition, results of the current study are in agreement with the results of Nandal & Bhatti[19] who have reported that some of the plant extracts showed significant nematicidal properties. According to Khan,[20] many wild and cultivated medicinal plants have been shown to possess nematicidal properties against several plant-parasitic nematodes. The results of the study showed that neem extract had a toxic effect on the root-knot nematode *in vitro* by inhibiting the egg hatching at different concentrations of the extract. It was also observed that inhibition of egg hatching increased with increasing the concentration of the extract with the highest score that was recorded with the extract concentration of 4000ppm. This observation agrees with the findings of Adegbite and Adesiyan[21] working with root extracts of *Azadirachtaindica*, *Chromolaenaodorata*, *Ricinuscommunis* and *Jatrophacurcas* and recorded the gradual increase in inhibition of egg hatching with increasing the concentration of the extract. A similar

finding was reported by Ameer-Zareen et al.,[22] on root-knot nematode eggs *in vitro* when they have used the aqueous extract of ginger (*Zingiberofficinale*). This study also agrees with the results of Barker[23] that nematode egg hatching was influenced by the exudates from its environment. In addition, egg hatching inhibition was increased with increase in exposure time, and this result also agrees with the results of Joymatti et al.,[24] The inhibitory effect of plant extracts on egg hatching of nematode according to Adegbite & Adesiyan,[21] might be due to the properties of the chemical compounds present in the extract that possess ovicidal properties. It was also suggested that botanicals with nematicidal properties affect the embryonic development or kill the eggs. Presumably, these properties found to increase with an increase in time, hence, the inhibition of egg hatching tend to increase with increasing the exposure period to the extract. These active chemicals either affect the embryonic development or kill the eggs or even dissolve the egg masses. It has been reported by Adegbite,[25] Goswami et al.,[26] and Hackney et al.,[27] that extracts (i.e., Siam weed; *Chromolaenaodorata* L., Neem; *Azadirachtaindica* A. Jass, Castor bean; *Ricinuscommunis* L., and Lemon grass; *Cymbopogoncitratrus* DC.) that contained alkaloids, flavonoids, saponins, amides including benzamide and ketenes in a single form or in a combination inhibited nematode egg hatching. From the results of the present study, it has been found that neem extract was recorded the best results regarding inhibition of egg hatching compared to moringa, lantana, and liquorices extracts. Therefore, neem extract was exposed to GC/MS analysis to find out the active chemical compounds (Figure 9) that caused the toxicity effect on nematodes. The active chemical compounds found in this outbalanced extract and identified it as a best effective extract in inhibition of egg hatching are alkaloids, flavonoids, saponins, amides including benzamide and ketones, and others as follows: Hexane, 2, 4-dimethyl; Hexane, 2,2,5-trimethyl-;Cyclohexane, 2,4-diethyl-1-methyl-; Methylbicyclo [4.2.0] octane; Methallylcyclohexane; Cyclohexane ethyl-; Heptane, 2,6-dimethyl; Cyclohexane, 1,2,4-trimethyl-, (1α,2β,4β)-; Trans-1,2-Diethyl cyclopentane; Octane, 4- methyl-; Heptane, 2,3-dimethyl-; Benzene, 1,3-dimethyl-; Cyclohexane, 1,1,2-trimethyl-; Cyclopentane, 1-methyl-2-propyh-; 1-Ethyl-4-methylcyclohexane; p-Xylene; Nonane; Cyclohexane, 1-ethyl-4-methyl-, cis-; Benzene (1-methylethyl)-; Cyclohexane, propyl-; Octane, 2,6-dimethyl-; Benzene, propyl-; Heptane, 3-ethyl-2-methyl-; Benzene, 1-ethyl-2- methyl-; Benzene, 1,2,3-trimethyl-; Decane; Decane, 4-methyl-; Decane, 2-methyl, Undecane; Undecane, 2-methyl-; Dodecane; -; o-Xylene; Mesitylene; and Naphthalene, decahydro-, trans-. Figure 9 expresses the presence of bioactive compounds which had been detected in petroleum ether extract of *Azadirachtaindica* during GC–MS analysis. According to Sharma et al.,[28] 4-hydroxy-4-methyl-2-pentanone, hexadecenoic acid (palmitoleic acid), hexadecanoic acid (palmitic acid), pentadecanoic acid, octadecene, diethylhexyl phthalate, cyclohexadiene-1-one and 1,2-benzenedicarboxylic acid were identified as main compounds from the rhizobacteria *Pseudomonas jessenii* strain R62 and *Pseudomonas synxantha* strain R81 extracts that had toxic effects on against root-knot nematode, *Meloidogyne incognita* second stage juveniles (J2). Many of these active chemical components were discovered in the extract of our study and showed toxic effects on egg hatching of *Meloidogyne incognita*. Our study is also in accordance, partially, with the others[29–30] where they reported that the ethyl acetate extract caused 64% inactivity in *Meloidogynejavanica* within 24 h' exposure and assumed that the active compound might be of *proteinaceous* or *glycoproteinaceous* in nature. Likewise, Padgham & Sikora[31] reported that *Bacillus megaterium* cause repellence of *M. graminicola* from rice roots. Adam et al.,[32] reported combined effect of bacteria produced systemic resistance in tomato due to the presence of nematicidal compounds. In GC–MS analysis large numbers of compounds were identified; some of these compounds are already known to be bioactive. Dibutyl phthalate,[33] heneicosane[34] and pentadecanoic acid[35] are reported as antimicrobial in nature. Hexadecenoic acid and hexadecanoic acid identified in the bioactive extracts were found to be active against nematode.[33] 4-Hydroxy4-methyl-2-pentanone has been reported to possess antibiotic,[36] while 2,4- di-ter-butyl-phenol showed antioxidant activity.[37–40] Many of the compounds found in the extract of *Azadirachtaindica* also remained unidentified in GC–MS analysis due to similar fragmentation pattern of many compounds with different retention times. It has been concluded from the results of the present study that the leaf extract of *Azadirachtaindica* has the ability to inhibit the egg hatchability of root-knot nematode. Thus, this finding is important in the identification and development of alternative strategies in controlling the root-knot nematodes. There is, however, further work is needed to identify some of these main compounds after purification. This study confirms the presence of nematicidal compounds in petroleum ether fractions of *Azadirachtaindica*, which were responsible for the prevention of egg hatching of root-knot nematodes at some concentrations, especially 4000ppm.

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Conflict of interest

The author declares there is no conflict of interest.

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